Cell Metabolism
Short Article

TRIAP1/PRELI Complexes Prevent Apoptosis by Mediating Intramitochondrial Transport of Phosphatidic Acid

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SUMMARY

Cardiolipin (CL), a mitochondria-specific glycerophospholipid, is required for diverse mitochondrial processes and orchestrates the function of various death-inducing proteins during apoptosis. Here, we identify a complex of the p53-regulated protein TRIAP1 (p53CSV) and PRELI in the mitochondrial intermembrane space (IMS), which ensures the accumulation of CL in mitochondria. TRIAP1/PRELI complexes exert lipid transfer activity in vitro and supply phosphatidic acid (PA) for CL synthesis in the inner membrane. Loss of TRIAP1 or PRELI impairs the accumulation of CL, facilitates the release of cytochrome *c*, and renders cells vulnerable to apoptosis upon intrinsic and extrinsic stimulation. Survival of TRIAP1- and PRELI-deficient cells is conferred by an excess of exogenously provided phosphatidylglycerol. Our results reveal a p53-dependent cell-survival pathway and highlight the importance of the CL content of mitochondrial membranes in apoptosis.

INTRODUCTION

Apoptosis is a highly regulated genetic program under the control of intrinsic and extrinsic signaling pathways, which converge on mitochondria. Signal transduction during programmed cell death depends on CL, a mitochondria-specific dimeric glycerophospholipid that affects apoptotic signaling in various ways (Schug and Gottlieb, 2009). CL retains cytochrome *c* at the mitochondrial inner membrane, promotes the oligomerization of BCL2-family members in the outer membrane, and triggers caspase-8 activation and formation of tBid during mitochondrial amplification of extrinsic apoptotic signals (Gonzalvez et al., 2008; Osman et al., 2011). Apoptosis is associated with extensive remodeling of cristae membranes and detachment of cytochrome *c* from CL facilitating its redistribution within the IMS and release from mitochondria (Iverson and Orrenius, 2004; Scorrano et al., 2002).

Apoptotic signaling is regulated at multiple levels by the sequence-specific transcription factor p53, with more than 100 genes containing p53 binding sites (Riley et al., 2008). However, the function of many of these genes remained enigmatic. This includes TP53-regulated inhibitor of apoptosis gene 1 (*TRIAP1* or *p53CSV*), which contains a p53 binding site within its coding sequence and is upregulated in multiple myeloma (Felix et al., 2009; Park and Nakamura, 2005). TRIAP1 has been shown to inhibit apoptosis potentially by interacting with cytoplasmic Hsp70 and Apaf1 (Park and Nakamura, 2005) and recently was identified as a repressor of p21 (Andrysik et al., 2013), but the precise mechanism of TRIAP1 function remained unaddressed.

TRIAP1 attracted our attention because it is homologous to yeast Mdm35, a regulator of mitochondrial phospholipid biogenesis (Potting et al., 2010; Tamura et al., 2010). Here, we identify TRIAP1 and PRELI as subunits of an intramitochondrial lipid transfer complex promoting CL accumulation and linking p53-mediated cell survival to mitochondrial CL.

RESULTS

TRIAP1 Modulates the Mitochondrial Pathway of Apoptosis

We depleted HeLa cells of TRIAP1 by siRNA-mediated downregulation and assessed the susceptibility of the cells toward actinomycin D (ActD)-induced apoptosis (Figure 1A and see Figure S1 online). In agreement with previous findings in UV-treated cells (Park and Nakamura, 2005), we observed an increased rate of apoptosis specifically in TRIAP1-depleted cells, as monitored by the accumulation of cleaved poly ADP-ribose polymerase (cPARP; Figure 1A; see Figure 4E for quantification). Transient expression of $TRIAP1^{HA}$ harboring a C-terminal HA-tag partially prevented the formation of cPARP (Figure 1A). Moreover, we treated TRIAP1-depleted cells with TNF-α/CHX to induce apoptosis extrinsically. cPARP accumulated faster in cells depleted of TRIAP1 than in control cells, suggesting a function for TRIAP1 in both intrinsic and extrinsic pathways of apoptosis (Figure 1B; see Figure 4H for quantification).

These findings prompted us to examine a potential role of mitochondria in TRIAP1-dependent apoptosis. Upon ActD treatment, cytochrome *c* accumulated in the cytosol in a significantly increased number of TRIAP1-depleted cells (Figure 1C; see Figure 4B for quantification), indicating that the loss of TRIAP1 facilitates the release of cytochrome *c* from mitochondria.

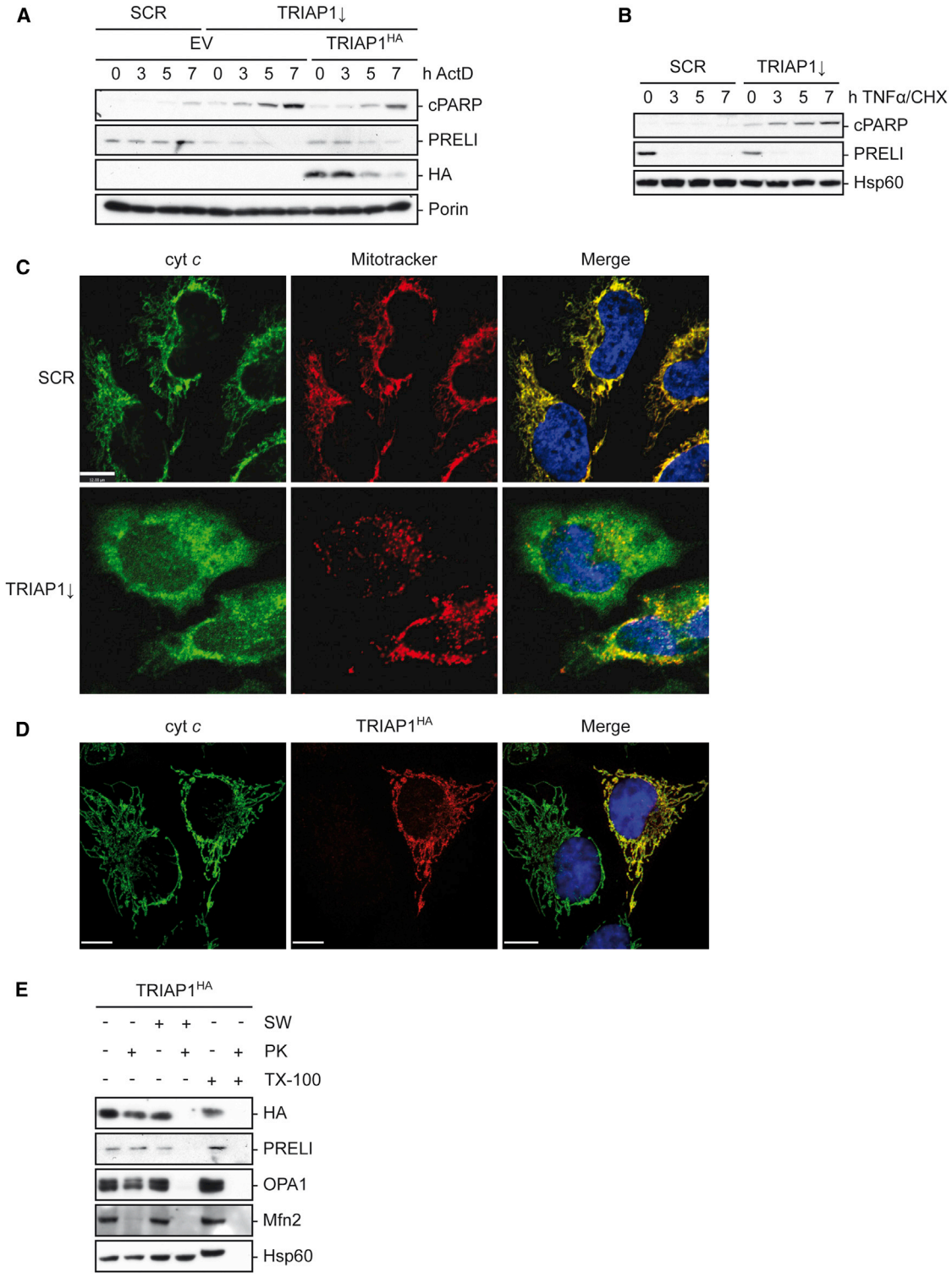

Figure 1. Mitochondrial TRIAP1 Protects against Apoptosis

(A) Increased susceptibility of TRIAP1-deficient cells toward actinomycin D (ActD)-induced apoptosis. HeLa cells were transfected with scrambled (SCR) or *TRIAP1*-specific siRNAs and either a control plasmid (EV) or a plasmid that contains siRNA-resistant *TRIAP1* encoding HA-tagged TRIAP1 (TRIAP1^{HA}). After induction of apoptosis with ActD (0.05 μM), cells were harvested at the indicated time points and analyzed by SDS-PAGE and immunoblotting. See Figure 4E for quantification. PRELI levels are decreased upon downregulation of TRIAP1, which is required for PRELI stability (see Figure 2). cPARP, cleaved PARP; HA, HA-specific antibodies.

(B) Increased susceptibility of TRIAP1-deficient cells toward apoptosis induced with TNF-α (0.2 ng/ml)/CHX (1 μg/ml). See Figure 4H for quantification.

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While TRIAP1 has been proposed to modulate apoptosis by interacting with cytosolic Hsp70 and Apaf1 (Park and Nakamura, 2005), its cellular localization remained to be clarified. Confocal immunofluorescence microscopy following ectopic expression of TRIAP1^{HA} in HeLa cells showed a reticulate distribution of TRIAP1^{HA} and colocalization with cytochrome *c*, suggesting a mitochondrial localization of TRIAP1 (Figure 1D). Consistently, TRIAP1^{HA} was present in mitochondria purified from these cells (Figure 1E). To determine the submitochondrial localization of TRIAP1, we performed protease protection experiments upon fractionation of mitochondria. TRIAP1^{HA} remained stable against externally added protease and was only degraded under hypotonic conditions, which result in the osmotic disruption of the outer membrane (Figure 1E). We conclude from these experiments that TRIAP1 is localized in the mitochondrial IMS.

TRIAP1 Assembles with PRELI in the IMS

Proteins homologous to TRIAP1 are present in all eukaryotic kingdoms (Figure 2A). The yeast member of this family, Mdm35, binds Ups1, which is homologous to PRELI (protein of relevant evolutionary and lymphoid interest) that we found to localize to the IMS as well (Figure 1E) (Dee and Moffat, 2005; Fox et al., 2004). In agreement with previous findings (McKeller et al., 2010), downregulation of PRELI increased the susceptibility of HeLa cells toward apoptosis upon treatment with ActD or TNF-α/CHX, as indicated by augmented PARP cleavage and facilitated cytochrome *c* release from mitochondria (Figures 2B–2D; see Figure 4 for quantification).

PRELI was rapidly degraded upon induction of apoptosis (Figures 2B and 2C). Expression of a catalytically inactive variant of the *i*-AAA protease YME1L, harboring a mutation in the Walker B motif of the AAA ATPase domain (YME1L^{E381Q}), led to the accumulation of PRELI (Figure 2E). Similarly, siRNA-mediated downregulation of YME1L stabilized PRELI, indicating proteolysis of PRELI by YME1L (Figure 2F). Notably, the steady-state level of PRELI was dependent on TRIAP1. PRELI accumulated upon overexpression of TRIAP1^{HA} in YME1L-depeleted mitochondria (Figure 2F), while it was present at reduced levels in mitochondria lacking TRIAP1 (Figure 2G). Thus, it appears that TRIAP1 protects PRELI against YME1L-mediated proteolysis, suggesting a physical interaction between TRIAP1 and PRELI.

To test this hypothesis, we transiently expressed TRIAP1^{HA} in cells depleted of YME1L and performed coimmunoprecipitation experiments using HA-specific antibodies. PRELI was specifically precipitated with TRIAP1^{HA} (Figure 2H). Furthermore, upon size exclusion chromatography of mitochondrial extracts, endogenous TRIAP1 and PRELI coeluted from the column in fractions corresponding to a molecular weight of ~100 kDa, indicating complex formation of both proteins (Figure 2I). Consistently, when extracts of PRELI-depleted mitochondria were analyzed, TRIAP1 eluted in low-molecular-weight fractions, likely representing unassembled TRIAP1. We conclude from these experiments that TRIAP1 and PRELI form a complex in the intermembrane space (IMS) of mitochondria.

TRIAP1/PRELI Complexes Ensure the Accumulation of CL in Mitochondrial Membranes

How do TRIAP1/PRELI complexes affect the progression of apoptosis? PRELI had been proposed to modulate apoptosis affecting mitochondrial morphology by interaction with the dynamin-like GTPase OPA1 (McKeller et al., 2010). However, we detected tubular mitochondria in the majority of cells lacking TRIAP1 or PRELI and observed mitochondrial fragmentation in only a minority of PRELI-deficient cells (Figures 3A and 3B). On the other hand, the basal oxygen consumption, as well as electron transfer capacity, was significantly decreased in PRELI-depleted cells (Figure 3C). Moreover, fewer respiratory supercomplexes containing cI, cIII, and cIV accumulated in TRIAP1-depleted and, more drastically, PRELI-depleted mitochondria (Figure 3D).

Since both the stability of respiratory supercomplexes and the apoptotic resistance of cells depend on CL (Osman et al., 2011), we assessed CL levels in TRIAP1- and PRELI-deficient mitochondria by mass spectrometry (MS). The loss of TRIAP1 resulted in a statistically significant reduction of mitochondrial CL but had only minor effects on other phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylglycerol (PG), or phosphatidic acid (PA) (Figure 3E). CL levels were more drastically decreased in PRELI-depleted mitochondria (Figure 3F). Moreover, PG, which serves as a precursor lipid for the synthesis of CL, was decreased in these cells, while PE levels were slightly increased and may partially compensate for the loss of CL (Figure 3F). Thus, the loss of PRELI, and to some extent of TRIAP1, causes significant dysregulation of the lipid composition of mitochondrial membranes, with the most prominent effect on the accumulation of CL.

TRIAP1/PRELI Complexes Facilitate the Transport of PA In Vitro

CL is synthesized along an enzymatic cascade in the IM (Chicco and Sparagna, 2007; Claypool and Koehler, 2012; Houtkooper and Vaz, 2008). The observation of reduced CL levels in TRIAP1- and PRELI-deficient mitochondria raised the intriguing possibility that TRIAP1/PRELI complexes act as lipid transfer proteins in the IMS of human mitochondria, as was recently demonstrated for Mdm35/Ups1 complexes in yeast (Connerth et al., 2012). We therefore purified TRIAP1/PRELI complexes after coexpression of both proteins from *E. coli* and assessed their ability to transport phospholipids between liposomes in vitro (Figure S2B). TRIAP1/PRELI complexes facilitated the transfer of PA, a CL precursor phospholipid, in a concentration-dependent manner,

(C) Increased cytochrome *c* release upon downregulation of TRIAP1. Apoptosis was induced with ActD (0.2 μM) in the presence of caspase inhibitor (Z-VAD [OME]-FMK). After 6 hr, cells were stained with mitotracker, DAPI, and cytochrome *c*-specific antibodies. Scale bar, 12 μm.
(D) TRIAP1^{HA} is localized in mitochondria. HeLa cells transiently expressing TRIAP1^{HA} were immunostained with HA- and cytochrome *c*-specific antibodies. Scale bar, 10 μm.
(E) TRIAP1^{HA} localizes to the IMS. Mitochondria harboring TRIAP1^{HA} were subjected to osmotic swelling (SW) or Proteinase K treatment (PK; 50 μg/ml) or were solubilized with Triton X-100 (TX-100) as indicated.
See also Figure S1.

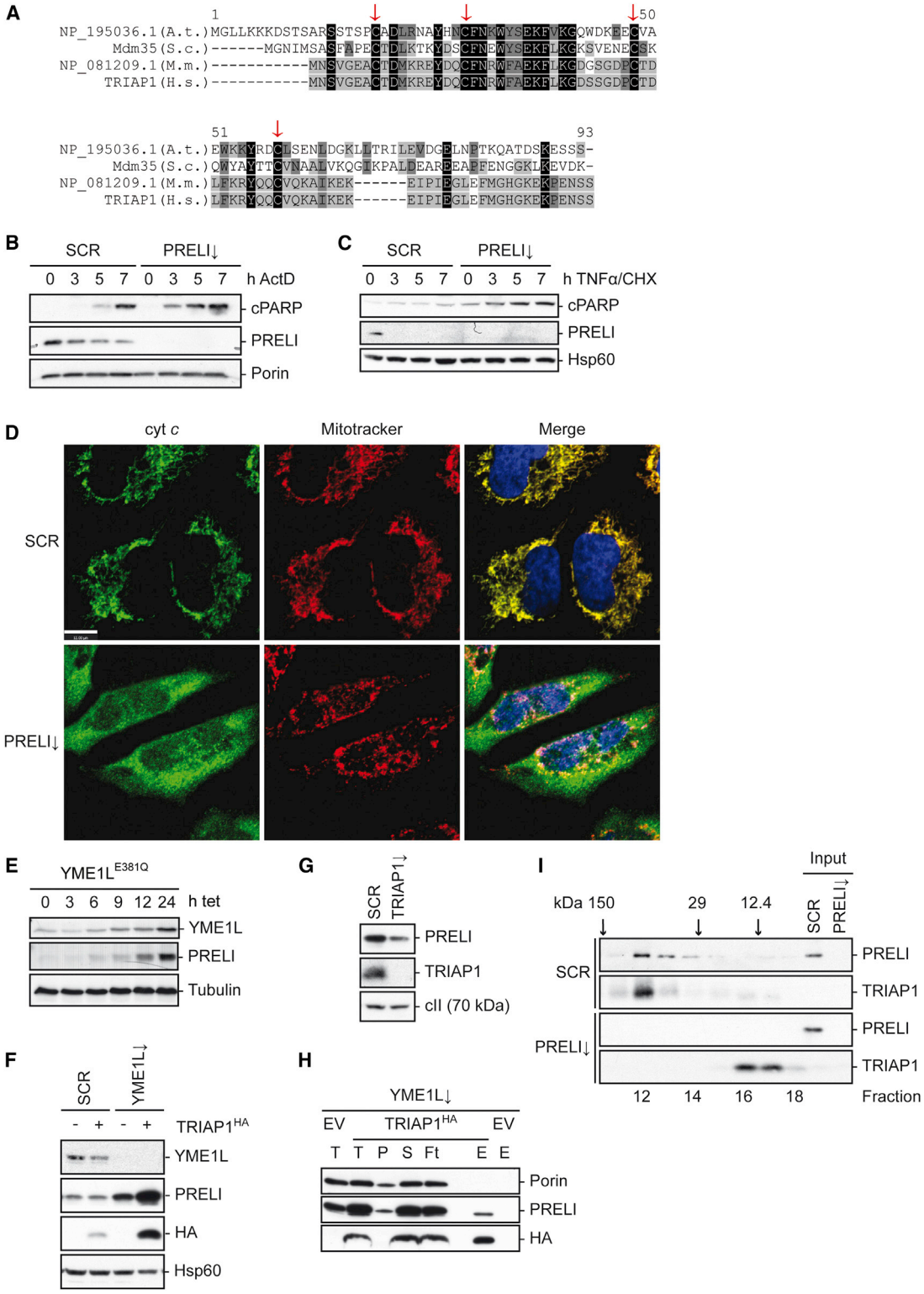

Figure 2. TRIAP1 Assembles with PRELI in the Intermembrane Space of Mitochondria

(A) Multiple sequence alignment of *Saccharomyces cerevisiae* (S.c.) Mdm35, *Homo sapiens* (H.s.) TRIAP1, and homologs from *Mus musculus* (M.m.) and *Arabidopsis thaliana* (A.t.). Amino acids conserved across species are highlighted in black, similar amino acids are highlighted in dark gray, and light gray highlighting indicates conservation in at least two species. Red arrows point to the twin Cx_9C motif.

(B and C) Increased susceptibility of PRELI-deficient cells toward apoptosis induced with ActD (0.05 μM) (B) or TNF-α (0.2 ng/ml)/CHX (1 μg/ml) (C). See Figures 4E and 4H for quantification.

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while PI, PS, PG, and CL were not transported (Figure 3G). We observed some transport of CDP-diacylglycerol (CDP-DAG) by TRIAP1/PRELI complexes, albeit with drastically lower efficiency (Figure 3G). To substantiate these findings, we devised a fluorescence-based lipid transfer assay using NBD-PA (Figure 3H). Donor liposomes containing NBD-PA and rhodamine-PE that quenches NBD fluorescence were incubated with acceptor liposomes lacking fluorescent lipids. Dequenching of NBD fluorescence allows monitoring of the transfer of NBD-PA. TRIAP1/PRELI complexes accelerated dequenching of NBD-PA in a concentration-dependent manner (Figure 3I). Only trace amounts of rhodamine fluorescence were detected in isolated acceptor liposomes after the reaction excluding transfer of rhodamine-PE or liposome fusion (data not shown).

We conclude that TRIAP1/PRELI complexes mediate the transfer of PA between liposomes in vitro. Therefore, the reduced CL levels in TRIAP1- or PRELI-deficient mitochondria likely result from an impaired PA transport limiting CL synthesis.

PG Restores Apoptotic Resistance of Cells Lacking TRIAP1 or PRELI

To demonstrate directly that reduced CL levels cause the decreased apoptotic resistance of cells lacking either TRIAP1 or PRELI, we supplied these cells with excess PG to artificially restore CL-dependent functions in mitochondria. PG can at least partially substitute for CL functions, e.g., in binding cytochrome *c* (Chang et al., 1998; Rytömaa and Kinnunen, 1994). After incubation of cells with NBD-PG and a lipid-based transfection reagent, cellular lipid extracts were analyzed by thin-layer chromatography (TLC). Fluorescent signals indicated uptake of NBD-PG by the cells (Figure 4A). We observed multiple fluorescent bands suggesting that NBD-PG is metabolized and contributes to the biogenesis of distinct lipid species. Although prominent species remain to be identified, we observed comigration of fluorescent signals with CL, which were slightly reduced in extracts from cells transfected with cardiolipin synthase (CRLS1)-specific siRNAs, therefore likely representing NBD-CL (Figure 4A; see Figure S3A for quantification). Notably, although NBD-PG was taken up with similar efficiency, synthesis of NBD-CL was significantly enhanced in PRELI-depleted cells (Figure 4A). Thus, externally added PG can be taken up by the cells and reaches the mitochondrial inner membrane where it is, at least to a minor extent, converted into CL.

Next, we assessed the apoptotic resistance of TRIAP1- and PRELI-deficient cells that had received PG treatment. PG accumulated in lipid extracts of these cells, while CL levels remained largely unaffected (Figure S3B). When incubated with ActD, we observed a marked retention of mitochondrial cytochrome *c* in both TRIAP1- and PRELI-deficient cells containing excess PG, but not in control cells (Figure 4B and Figure S3C). Moreover, PARP cleavage was significantly reduced in these cells (Figures 4C–4E). Similarly, addition of excess PG protected cells lacking TRIAP1 or PRELI against TNF-α/CHX-induced apoptosis (Figures 4F–4H) but did not affect the formation of cPARP in ActD- or TNF-α/CHX-treated control cells (Figures S3D and S3E). These experiments demonstrate that excess PG partially restores the apoptotic resistance of TRIAP1- and PRELI-deficient cells.

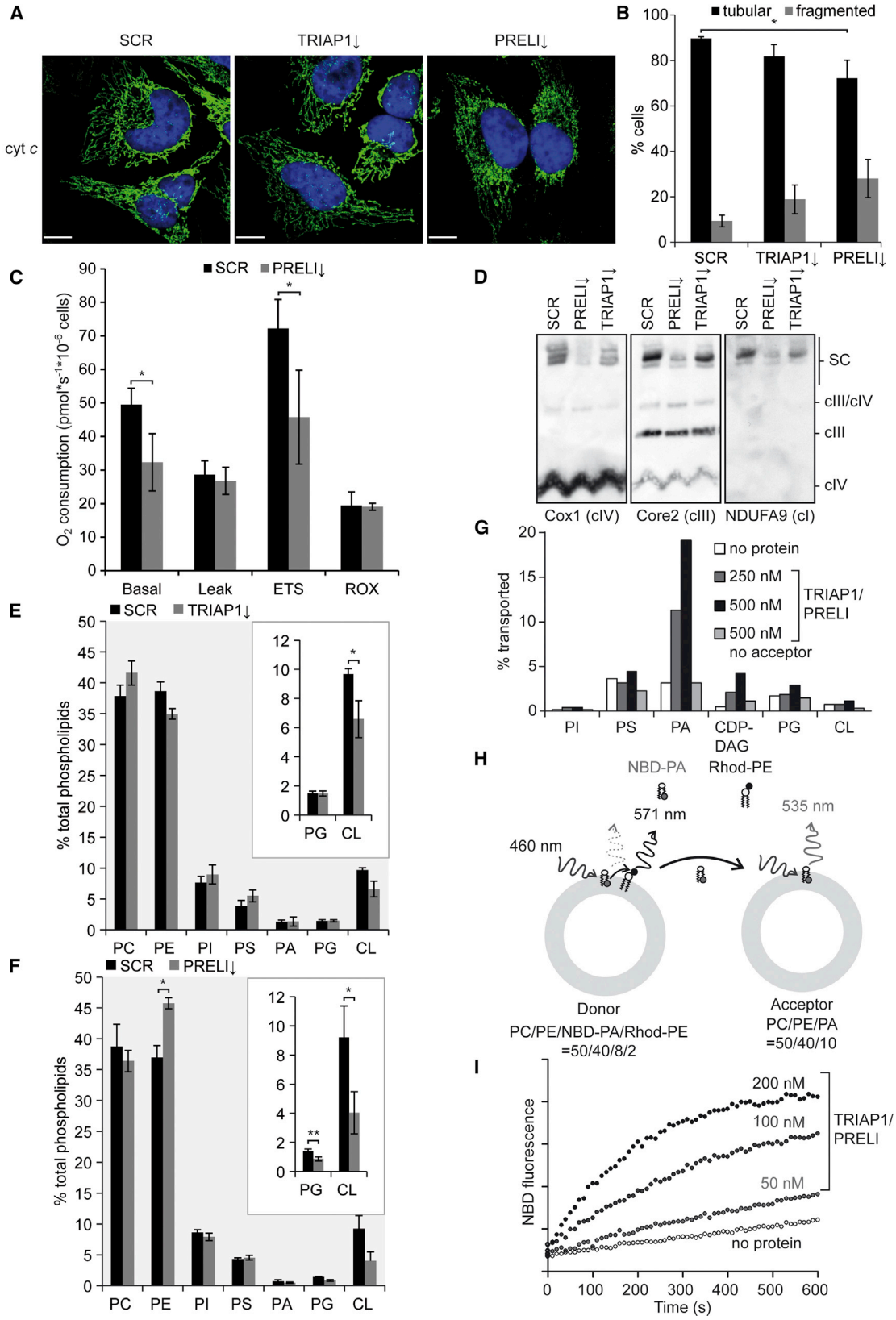
DISCUSSION

TRIAP1 and PRELI were previously shown to be required for apoptotic resistance (McKeller et al., 2010; Park and Nakamura, 2005), but the molecular role of either protein remained obscure. We demonstrate here that TRIAP1 and PRELI assemble into a lipid transfer complex in mitochondria, which mediates the transport of PA across the IMS and provides PA for CL synthesis in the inner membrane. Decreased CL levels in the absence of TRIAP1 or PRELI facilitate the release of cytochrome *c* from mitochondria, rendering cells vulnerable to apoptosis.

Purified TRIAP1/PRELI complexes transfer PA between liposomes in vitro, demonstrating lipid transfer activity. They exert a high specificity for PA, although we observed TRIAP1/PRELI-dependent transport of CDP-DAG with low efficiency. The assembled TRIAP1/PRELI complex likely represents the functionally active structure not only in vitro but also in vivo, as we observed reduced steady-state levels and proteolysis of PRELI in TRIAP1-depleted cells. Therefore, the loss of the assembled complex likely causes deficiencies in the absence of either PRELI or TRIAP1. The function of TRIAP1/PRELI complexes as PA transfer proteins is evolutionary conserved from yeast to man (Connerth et al., 2012). Notably, Mdm35 assembles with different members of the Ups1-family in yeast, affecting the accumulation of other phospholipids like PE in mitochondrial membranes (Potting et al., 2010; Tamura et al., 2010). Although the majority of TRIAP1 appears to assemble with PRELI, the functional conservation of TRIAP1/PRELI complexes opens up the possibility that depending on cell type or physiological conditions TRIAP1 also assembles with human homologs of PRELI into independent lipid transfer complexes.

We observed only a partial reduction in the CL levels in TRIAP1- and PRELI-deficient mitochondria, which might be explained by a limited knockdown efficiency in these experiments. Alternatively, other pathways may exist, which provide PA to the CL biosynthetic cascade if PA cannot be transported across the IMS. Mitochondria harbor the acylglycerol kinase AGK, which is mutated in Sengers syndrome (Mayr et al.,

(D) Loss of PRELI facilitates cytochrome *c* release from mitochondria upon ActD (0.2 μM) treatment. See Figure 1C. Scale bar, 12 μm.
(E) Accumulation of PRELI upon tetracycline-induced, stable expression of proteolytically inactive YME1L^{E381Q} in HEK293 cells.
(F) Mitochondrial accumulation of PRELI upon downregulation of YME1L in HeLa cells, transiently expressing TRIAP1^{HA} when indicated. Transfection efficiency was higher in YME1L-depleted cells as monitored by cotransfection of a plasmid carrying EGFP (data not shown). HA, HA-specific antibodies.
(G) Reduced steady-state level of PRELI in TRIAP1-deficient mitochondria.
(H) PRELI assembles with TRIAP1^{HA}. Mitochondria depleted of YME1L and harboring either a control plasmid (EV) or a plasmid encoding TRIAP1^{HA} were solubilized with digitonin, and coimmunoprecipitation was performed using HA-specific antibodies. Total (T), pellet (P), supernatant (S), flowthrough (Ft) fractions (5%), and the eluate (E; 100%).
(I) Mitochondria from cells treated with indicated siRNAs were solubilized with dodecylmaltoside and fractionated by size exclusion chromatography. TRIAP1 is only detected in the input fraction upon long exposure of the gel.



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2012). AGK is a multisubstrate lipid kinase that catalyzes the phosphorylation of diacylglycerol or monoacylglycerol to PA or lyso-PA, respectively, and therefore has been proposed to contribute to CL synthesis (Bektas et al., 2005; Mayr et al., 2012). Moreover, genetic evidence in yeast points to alternative CL synthesis routes (Connerth et al., 2012; Tamura et al., 2013). While components involved in this pathway remain to be identified, our experiments provide evidence for the existence of an additional CL synthesis pathway in human mitochondria. CL synthesis from exogenously added PG was strongly increased upon downregulation of PRELI, suggesting that the impaired transport of PA across the IMS stimulates CL synthesis from PG along another pathway.

CL is critical for a variety of cellular processes and essential for embryonic development in mice (Zhang et al., 2011). Loss of CL, an altered acyl chain composition of CL, and/or CL peroxidation has been linked to aging and a variety of pathophysiological conditions, including ischemia, heart failure, and cardiomyopathies (Chicco and Sparagna, 2007; Claypool and Koehler, 2012; Houtkooper and Vaz, 2008). While the general importance of CL and CL peroxidation for apoptotic signaling has been recognized (Kagan et al., 2005; Schug and Gottlieb, 2009), anti- and proapoptotic effects have been observed upon downregulation of the CL synthase CRLS1 (Choi et al., 2007; Huang et al., 2008). Our results establish the protective effect of CL against the release of cytochrome *c* and apoptosis. Silencing of TRIAP1 or PRELI impairs the accumulation of CL and increases the susceptibility of these cells to apoptosis. The restoration of the apoptotic resistance of these cells by excess PG demonstrates a direct link between CL dysregulation and apoptosis. Thus, similar to CL peroxidation, the loss of CL facilitates cytochrome *c* release and apoptotic signaling.

The central role of TRIAP1 within mitochondrial CL metabolism and apoptotic signaling sheds new light on the regulation of *TRIAP1* expression by p53. TRIAP1 allows cell-cycle progression (Andrysik et al., 2013) and is upregulated in multiple myeloma (Felix et al., 2009), and low genotoxic stress causes p53-mediated expression of *TRIAP1* and increased cell survival (Park and Nakamura, 2005). On the other hand, PRELI has been identified as a regulator of lymphocytic apoptosis and differentiation (McKeller et al., 2010; Tahvanainen et al., 2009). Our results explain these observations by the role of TRIAP1/PRELI complexes in intramitochondrial PA trafficking and CL synthesis.

EXPERIMENTAL PROCEDURES

siRNA Experiments and Transfections

siRNA-mediated downregulation was performed using specific or scrambled siRNAs (SCR) and Lipofectamine RNAiMax (Life Technologies) according to the manufacturer's instructions. Cells were grown for 2 days, and, if not indicated otherwise, media was replaced followed by 12 hr incubation prior to harvesting. Downregulation efficiency was assessed by immunoblotting or qRT-PCR.

For expression of siRNA-resistant TRIAP1^{HA}, human *TRIAP1* cDNA was cloned into pcDNA5FRT/TO (Invitrogen). Silent mutations were introduced into the siRNA target sequence, and the HA-tag coding sequence was inserted before the stop codon by PCR. Transfections were performed using the GeneJuice transfection reagent (Novagen) according to the manufacturer's instructions.

Analysis of Apoptotic Resistance

Adherent cells were washed in PBS before adding fresh media supplemented with ActD or TNF-α/CHX as indicated. Cells were harvested at indicated time points, lysed, and analyzed by SDS-PAGE and immunoblotting as indicated.

Increase of Cellular PG Levels

Cells were seeded in standard growth media and transfected essentially according to manufacturer's instructions using PG-supplemented Optimem media (Optimem [Life Technologies] supplemented with 0.15 mM PG- or NBD-PG). PG-supplemented Optimem media was sonicated prior to use in transfections. After 2.5 days, adherent cells were washed in PBS, and fresh media was added. For whole-cell lipid analyses, cells were harvested and lipids were analyzed by MS or by TLC.

Monitoring Phospholipid Transfer In Vitro

MS analysis of lipids and lipid transfer assays were performed essentially as described (Connerth et al., 2012). Donor liposomes (25 μM), whose lipid composition resembled the OM (PC/PE/PI/CL/PA/PG/PS/CDP-DAG/NBD-PE/N-lactosyl-PE = 40%/9.6%/5%/5%/5%/5%/5%/%5/0.4/10%) and acceptor liposomes (100 μM; PC/PE/Rhod-PE = 50%/49.9%/0.1%) were incubated with indicated concentrations of TRIAP1/PRELI complexes. Acceptor membranes were isolated by flotation, and phospholipids were quantified by MS. To monitor the kinetics of the transfer reaction, donor liposomes (12.5 μM; PC/PE/18:1–12:0 NBD-PA/Rhod-PE = 50%/40%/8%/2%) and acceptor liposomes (50 μM; PC/PE/PA = 50%/40%/10%) were incubated with TRIAP1/PRELI complexes, and NBD fluorescence was monitored.

Miscellaneous

Further experimental procedures can be found in the Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes three figures, Supplemental Experimental Procedures, and Supplemental References and can be found with this article online at http://dx.doi.org/10.1016/j.cmet.2013.07.008.

Figure 3. The Accumulation of CL in Mitochondria Depends on TRIAP1 and PRELI

(A) Mitochondrial morphology in TRIAP1- and PRELI-deficient cells. Cells transfected with the indicated siRNAs were stained with DAPI and cytochrome *c*-specific antibodies. Scale bar, 10 μm.

(B) Statistical evaluation of mitochondrial morphologies. More than 200 cells were analyzed. n = 3. Error bars represent SD values. *p < 0.05.

(C) Oxygen consumption rates of intact cells transfected with indicated siRNAs. The basal rate, the complex V inhibited (Leak) rate, and the maximal electron transfer system capacity (ETS) are shown. Inhibition of electron flow through complex I and III demonstrates that nonmitochondrial residual oxygen consumption (ROX) is unaffected by PRELI downregulation. n = 3. Error bars represent SD values. *p < 0.05.

(D) Impaired formation of respiratory supercomplexes in PRELI-deficient mitochondria. Mitochondria were isolated from HeLa cells transfected with the indicated siRNAs, solubilized with 1.5% digitonin (6 g/g protein) and analyzed by blue native gel electrophoresis and immunoblotting with antibodies directed against complexes (c) I, III, and IV. SC, respiratory supercomplexes (Acín-Pérez et al., 2008). Note that lower signals in the cIII panel represent residual cIV signals.

(E and F) Reduced CL in TRIAP1- (E) and PRELI-deficient (F) mitochondria. Mitochondria were purified from siRNA-treated HeLa cells (see Figure S2A for assessment of the purity of mitochondria); lipids were extracted and quantified by MS. n = 3. Error bars represent SD values. *p < 0.05; **p < 0.01.

(G) Phospholipid transfer by TRIAP1/PRELI complexes in vitro.

(H) Schematic representation of the lipid transfer assay using NBD-PA.

(I) Transport of NBD-PA by TRIAP1/PRELI complexes.

See also Figure S2.

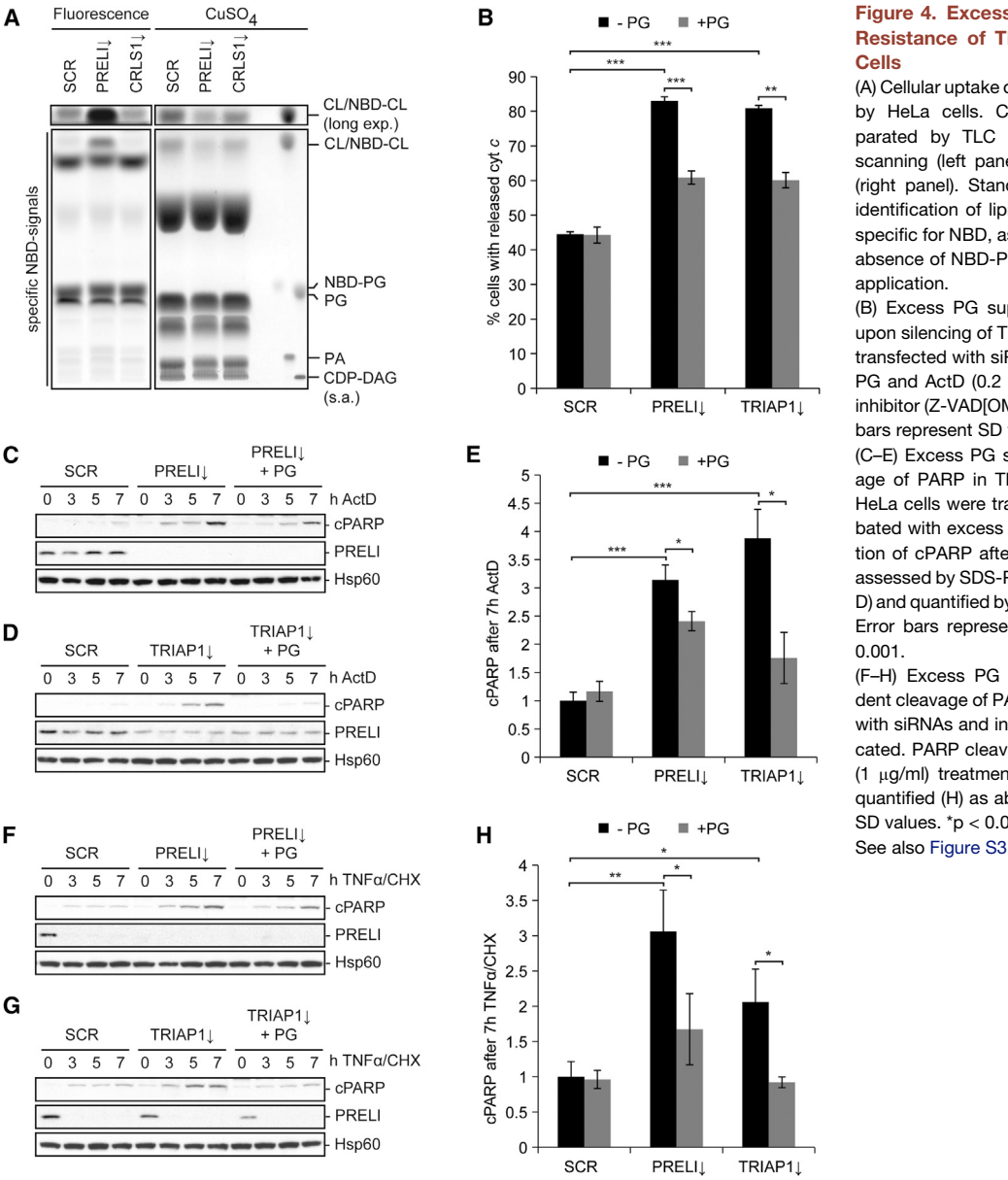

Figure 4. Excess PG Restores the Apoptotic Resistance of TRIAP1- and PRELI-Deficient Cells

(A) Cellular uptake of exogenously supplied NBD-PG by HeLa cells. Cellular phospholipids were separated by TLC and analyzed by fluorescence scanning (left panel) prior to staining with $CuSO_4$ (right panel). Standard phospholipids allowed the identification of lipid spots. Fluorescent bands are specific for NBD, as no signals were detected in the absence of NBD-PG (data not shown). s.a., sample application.

(B) Excess PG suppresses cytochrome *c* release upon silencing of TRIAP1 or PRELI. HeLa cells were transfected with siRNAs and incubated with excess PG and ActD (0.2 μM) in the presence of caspase inhibitor (Z-VAD[OME]-FMK); >200 cells; n = 3. Error bars represent SD values. **p < 0.01; ***p < 0.001.

(C–E) Excess PG suppresses ActD-induced cleavage of PARP in TRIAP1- or PRELI-deficient cells. HeLa cells were transfected with siRNAs and incubated with excess PG as indicated. The accumulation of cPARP after ActD treatment (0.05 μM) was assessed by SDS-PAGE and immunoblotting (C and D) and quantified by infrared laser scanning (E). n = 3. Error bars represent SD values. *p < 0.05; ***p < 0.001.

(F–H) Excess PG suppresses TNF-α/CHX-dependent cleavage of PARP. HeLa cells were transfected with siRNAs and incubated with excess PG as indicated. PARP cleavage after TNF-α (0.2 ng/ml)/CHX (1 μg/ml) treatment was assessed (F and G) and quantified (H) as above. n = 3. Error bars represent SD values. *p < 0.05; **p < 0.01.

See also Figure S3.

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